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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Christopher J.	SOARES	La Jolla, California

☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

COMPOSITIONS AND METHODS FOR THE TREATMENT OF OBESITY

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☒ Customer Number 28381

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☐ Firm *or* Individual Name					
Address					
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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 39 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 2 ☒ Other (specify) 3 a Sequence Listing (3 pages)

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

FILING FEE AMOUNT ($)

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-2387 $160.00

☐ Payment by credit card. Form PTO-2038 is attached.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____.

Respectfully submitted,

SIGNATURE _____ DATE February 11, 2004

TYPED or PRINTED NAME David R. Marsh REGISTRATION NO. *(if appropriate)* 41,408

TELEPHONE (202) 942-5000 DOCKET NUMBER 18528.682

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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February 11, 2004

Mail Stop Provisional Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Re: New U.S. Provisional Application
Appln. No.: To Be Assigned
Filed: Herewith
For: *Compositions and Methods for the Treatment of Obesity*
Applicants: Christopher J. SOARES
Atty Docket: 18528.682

Sir:

The following documents are forwarded herewith for appropriate action by the U.S. Patent and Trademark Office (PTO):

1. Provisional Application Transmittal (PTO/SB/16);
2. U.S. Provisional Application entitled:

Compositions and Methods for the Treatment of Obesity

and naming as inventors:

Christopher J. SOARES;

the application consisting of:

a. a specification containing:

(i). 37 pages of a description prior to the claims;

(ii). 1 page of claims (1 claim);

(iii). a one (1) page abstract;

b. 2 pages of drawings (Figures 1 and 2); and

b. a paper copy of the sequence listing (3 pages);

3. two (2) return postcards.

Please stamp one of two attached postcards with the filing date of these documents and return it to our courier, and stamp the other prepaid postcard with the filing date and unofficial application number and return it as soon as possible.

Authorization to charge the provisional application filing fee to counsel's deposit account is given in the accompanying Form PTO/SB/16.

Applicants do not believe that any fees other than the provisional application filing fee are due in conjunction with this filing. However, should any additional fees be required, the Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to Arnold & Porter LLP Deposit Account No. 50-2387 referencing docket number 18528.682. A duplicate copy of this letter is enclosed.

Respectfully submitted,

David R. Marsh (Reg. No. 41,408)
Milan M. Vinnola (Reg. No. 45,979)

Attachments

COMPOSITIONS AND METHODS FOR THE TREATMENT OF OBESITY

TECHNICAL FIELD

The present invention relates to compositions and methods for treating conditions or disorders which can be alleviated by reducing caloric intake. The methods are useful for treating conditions or disorders, in which the reduction of caloric intake is of value, including obesity, Type II diabetes, eating disorders, and insulin-resistance syndrome.

BACKGROUND

Obesity is a chronic disease that is highly prevalent in modern society and is associated not only with a social stigma, but also with decreased life span and numerous medical problems, including adverse psychological development, reproductive disorders such as polycystic ovarian disease, dermatological disorders such as infections, varicose veins, Acanthosis nigricans, and eczema, exercise intolerance, diabetes mellitus, insulin resistance, hypertension, hypercholesterolemia, cholelithiasis, osteoarthritis, orthopedic injury, thromboembolic disease, cancer, and coronary heart disease. Rissanen et al., British Medical Journal, 301: 835-837 (1990).

Obesity, and especially upper body obesity, is a common and very serious public health problem in the United States and throughout the world. According to recent statistics, more than 25% of the United States population and 27% of the Canadian population are over weight. Kuczmarski, Amer. J. of Clin. Nut. 55:495S-502S (1992); Reeder et. al., Can. Med. Ass. J., 23:226-233 (1992). Upper body obesity is the strongest risk factor known for type II diabetes mellitus, and is a strong risk factor for cardiovascular disease and cancer as well. Recent estimates for the medical cost of obesity are $150,000,000,000 world wide. The problem has become serious enough that the surgeon general has begun an initiative to combat the ever increasing adiposity rampant in American society.

Much of this obesity induced pathology can be attributed to the strong association with dyslipidemia, hypertension, and insulin resistance. Many studies have demonstrated that reduction in obesity by diet and exercise reduces these risk factors dramatically.

Unfortunately these treatments are largely unsuccessful with a failure rate reaching 95%. This failure may be due to the fact that the condition is strongly associated with genetically inherited factors that contribute to increased appetite, preference for highly caloric foods, reduced physical activity, and increased lipogenic metabolism. This indicates that people inheriting these genetic traits are prone to becoming obese regardless of their efforts to combat the condition. Therefore, a new pharmacological agent that can correct this adiposity handicap and allow the physician to successfully treat obese patients in spite of their genetic inheritance is needed.

Existing therapies for obesity include standard diets and exercise, very low calorie diets, behavioral therapy, pharmacotherapy involving appetite suppressants, thermogenic drugs, food absorption inhibitors, mechanical devices such as jaw wiring, waist cords and balloons, and surgery. Jung and Chong, Clinical Endocrinology, 35; 11-20 (1991); Bray, Am. J. Clin. Nutr., 55: 538S-544S (1992). Protein-sparing modified fasting has been reported to be effective in weight reduction in adolescents. Lee et al., Clin. Pediatr., 31: 234-236 (April 1992). Caloric restriction as a treatment for obesity causes catabolism of body protein stores and produces negative nitrogen balance. Protein-supplemented diets, therefore, have gained popularity as a means of lessening nitrogen loss during caloric restriction. Because such diets produce only modest nitrogen sparing, a more effective way to preserve lean body mass and protein stores is needed. In addition, treatment of obesity would be improved if such a regimen also resulted in accelerated loss of body fat. Various approaches to such treatment include those discussed by Weintraub and Bray, Med. Clinics N. Amer., 73:237 (1989); Bray, Nutrition Reviews, 49:33 (1991).

Considering the high prevalence of obesity in our society and the serious consequences associated therewith as discussed above, any therapeutic drug potentially useful in reducing weight of obese persons could have a profound beneficial effect on their health. There is a need for a drug that will reduce total body weight of obese subjects toward their ideal body weight and help maintain the reduced weight level.

SUMMARY

The present invention relates, at least in part, to novel obesity related polypeptides (ORPs). The ORPs of the present invention will generally retain, at least in part, a biological activity similar to that of native human amylin, *i.e.*, the ORPs of the present invention will generally have amylin agonist-like activity. In a preferred embodiment, the ORPs of the present invention will exhibit amylin activity in the treatment and prevention of metabolic conditions and disorders.

Preferred ORPs are those having a potency in one of the assays described herein (preferably food intake or weight reduction assays) which is greater than the potency of amylin in that same assay.

In another aspect of the invention, methods for treating or preventing obesity are provided, wherein the method comprises administering a therapeutically or prophylactically effective amount of an ORP to a subject in need thereof. In a preferred embodiment, the subject is an obese or overweight subject. While "obesity" is generally defined as a body mass index over 30, for purposes of this disclosure, any subject, including those with a body mass index of less than 30, who needs or wishes to reduce body weight is included in the scope of "obese." Subjects who are insulin resistant, glucose intolerant, or have any form of diabetes mellitus (*e.g.*, type 1, 2 or gestational diabetes) can benefit from this method.

In yet another aspect of the invention, methods of reducing food intake, reducing nutrient availability, causing weight loss, treating diabetes mellitus, and improving lipid profile (including reducing LDL cholesterol and triglyceride levels and/or changing HDL cholesterol levels) are provided, wherein the methods comprise administering to a subject an effective amount of an ORP. In a preferred embodiment, the methods of the invention are used to treat or prevent conditions or disorders which can be alleviated by reducing nutrient availability in a subject in need thereof, comprising administering to said subject a therapeutically or prophylactically effective amount of an ORP. Such conditions and disorders include, but are not limited to, hypertension, dyslipidemia, cardiovascular disease, eating disorders, insulin-resistance, obesity, and diabetes mellitus of any kind.

The present invention also relates to pharmaceutical compositions comprising a therapeutically or prophylactically effective amount of at least one ORP of the invention, or a pharmaceutically acceptable salt thereof, together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in the delivery of the ORP.

These and other aspects of the invention will be more clearly understood with reference to the following preferred embodiments and detailed description.

CERTAIN PREFERRED EMBODIMENTS

Embodiment 1. An Obesity Related Polypeptide (ORP) comprising an amino acid sequence of any one of SEQ ID NOs: 1 through 27.

Embodiment 2. An Obesity Related Polypeptide (ORP) comprising an amino acid sequence of any one of SEQ ID NOs: 1 through 27 with one or more substitutions, insertions, deletions, or combinations thereof.

Embodiment 3. A method of reducing food intake in a subject desirous of reducing food intake or in need thereof, comprising administering to said subject an effective amount of an ORP according to embodiments 1 .

Embodiment 4. A method of reducing weight in a subject desirous of reducing weight or in need thereof, comprising administering to said subject an effective amount of an ORP according to embodiment 1.

Embodiment 5. A method of treating diabetes mellitus comprising administering to a subject in need thereof, a therapeutically effective amount of an ORP according to any of embodiment 1.

Embodiment 6. A method of treating or preventing conditions or disorders which can be alleviated by reducing nutrient availability in a subject in need thereof, comprising administering to said subject a therapeutically or prophylactically effective amount of an ORP according to embodiment 1.

Embodiment 7. The method according to embodiment 6, wherein the condition or disorder is selected from the group consisting of: hypertension, dyslipidemia, cardiovascular risk, an eating disorder, insulin-resistance, obesity, and diabetes mellitus.

Embodiment 8. The method according to any of embodiments 3-7, wherein the ORP is peripherally administered via a mode of administration selected from the group

consisting of: parenteral administration, oral administration, pulmonary administration, transmucosal administration.

Embodiment 9. The method according to any of embodiments 3-8, wherein from about 1 μg to about 5 mg of the ORP is administered in single dose, divided doses, or controlled continual release.

Embodiment 10. The method according to any of embodiments 3-9, wherein the ORP has a potency in at least one of a food intake or weight reduction than amylin.

Embodiment 11. A method of treating or preventing obesity comprising administering to an obese or overweight subject a therapeutically or prophylactically effective amount of an ORP according to embodiment 1.

Embodiment 12. The method according to embodiment 11, wherein said subject is insulin resistant or glucose intolerant.

Embodiment 13. The method according to embodiment 11, wherein said subject has diabetes mellitus.

Embodiment 14. A pharmaceutical composition comprising an ORP according to embodiment 1, and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 demonstrates a dose-dependent reduction in food consumption in over night fasted mice to doses of AC2306 (SEQ ID NO: 3) (Food Intake Assay).

Figure 2A illustrates a decreased body weight gain in fattened C57BI/6 (diet-induced obese, or DIO) mice with continuous peripheral infusion of either a vehicle, salmon calcitonin, AC2221 (SEQ ID NO: 2), AC163231 (SEQ ID NO: 10) or AC163234 (SEQ ID NO: 13) over a period of two weeks.

Figure 2B illustrates a reduction in caloric intake over the corresponding time period.

DETAILED DESCRIPTION

The present invention relates to Obesity Related polypeptides (ORPs) useful in the treatment and prevention of metabolic conditions and disorders. In a preferred

embodiment, the ORPs of the invention may have comparable or higher potency in the treatment and/or prevention of metabolic conditions and disorders, as compared to amylin.

As such, the present invention provides ORP compositions and methods of using them to reduce weight in a subject in need thereof or desirous of losing weight. These methods may be useful in the treatment of, for example, obesity, diabetes, including but not limited to type 2 or non-insulin dependent diabetes, eating disorders, insulin-resistance syndrome, and cardiovascular disease.

The section headings are used herein for organizational purposes only, and are not to be construed as in any way limiting the subject matter described.

Obesity Related Polypeptides

The present invention relates, at least in part, to novel Obesity Related Polypeptides. The polypeptides of the present invention will generally retain, at least in part, a biological amylin agonist-like activity. In a preferred embodiment, the ORPs of the present invention will exhibit amylin agonist-like activity in the treatment and prevention of metabolic conditions and disorders.

By "amylin agonist-like activity" is meant that the polypeptides demonstrate similar physiological characteristics as amylin, such as, for example, reducing food intake. For example, the polypeptides of the present invention are capable of binding to or otherwise directly or indirectly interacting with an amylin receptor or other receptor or receptors with which amylin itself may interact to elicit a biological response, in particular reducing food intake in a similar manner as amylin. Amylin agonists are described for example in commonly assigned United States Patents 6,610,824 and 5,686,411, and commonly assigned patent application Serial No. 454,533 (filed December 6, 1999), the entireties of which are incorporated herein by reference.

By "Amylin" is meant a polypeptide obtained or derived from any species. Thus, the term "Amylin" includes the human full length amino acid peptide, and species variations of Amylin, including e.g., murine, hamster, chicken, bovine, rat, and dog Amylin. In this sense, "Amylin" and "wild-type Amylin" or "native Amylin," i.e., unmodified Amylin, are used interchangeably.

As such, the present invention provides ORPs. In a preferred embodiment, the present invention provides polypeptides selected from the following:

c(KCNTATCATQRLANFLVRSSNNLTNVGSNTY-NH2) (SEQ ID NO: 1),
c(KCNTATCATQRLANELVRLQTYPRTNVGSNTY-NH2) (SEQ ID NO: 2),
c(CSNLSTCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 3),
c(KCNTATCVLGRLSQELHRLQTYPRTNTGSNTY)-NH2) (SEQ ID NO: 4),
Isocap-STAVL-(Aib)-K(formyl)-LSQEL-(Aib)-K(formyl)-LQTYPRTNTGSGTP-NH2 (SEQ ID NO: 5),
c(KCNTATCATQRLANALVHSSNNFGAILPSTNVGSNTY-NH2) (SEQ ID NO: 6),
c(KCNTATCATARLAAFLARSSGY-NH2) (SEQ ID NO: 7),
c(KCNTATCATQRLANFLVHSGNNFGAILSSTNVGSNTY-NH2) (SEQ ID NO: 8),
c(CNTATCATARLAAFLARS-NH2) (SEQ ID NO: 9),
c(KCNTATCVLGKLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 10),
c(KCNTATCVLGRLSQELHRLQTLPRTNTGSNTY-NH2) (SEQ ID NO: 11),
c(KCNTATCVLGRLSQELHRLQTYPPTNTGSNTY-NH2) (SEQ ID NO: 12),
c(KCNTATCVLGRLSQELHRLQTYPRTNVGSNTY-NH2) (SEQ ID NO: 13),
c(KCNTATCVLGRLSQELHRLQTLPPTNVGSNTY-NH2) (SEQ ID NO: 14),
c(KCNTATCVLGRLANFLHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 15),
c(ACNTATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 16),
c(KCATATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 17),
c(KCNAATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 18),
c(KCNTAACVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 19),
c(CANLSTCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 20),
Isocap-STAVLGRLSQELHRLQTYPRTNTGSNTY-NH2 (SEQ ID NO: 21),
c(CSNASTCVLGRLSQELHRLQTYPRTNTGSNTY-NH2 (SEQ ID NO: 22),
c(CSNLATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 23),
c(CSNLSACVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 24),
c(KCNTATCVLGRLSQELHKLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 25),
c(KCNTATCVLGRLSQELHRLQTYPRTNTGSGTP-NH2) (SEQ ID NO: 26), and
c(KCNTATCATQRLSQELHRLQTYPRTNTGSGTP-NH2) (SEQ ID NO: 27).

Alternatively, the ORPs of the present invention can also be the free acid form of any of SEQ ID NOs. 1 through 27 above.

The ORPs of the present invention also include ORP analogs.

By "ORP analog" is meant a polypeptide compound based on the sequence of the polypeptides of the present invention, but including one or more sequence modifications. The analog polypeptides of the invention may be obtained through chemical, biochemical, or recombinant technologies, or any combination thereof. Such modifications include, but are not limited to, substitutions, deletions, and/or insertions of at least one amino acid residue, with reference to the amino acid sequence of the ORPs of the present invention (SEQ ID NOs: 1 through 27). The analog polypeptides of the invention may also be further derivatized by chemical alterations such as amidation, glycosylation, acylation, sulfation, phosphorylation, acetylation, and cyclization. Such chemical alterations may be obtained through chemical or biochemical methodologies, as well as through in-vivo processes, or any combination thereof. Derivatives of the analog polypeptides of the invention may also include conjugation to one or more polymers or small molecule substituents. One type of polymer conjugation is linkage or attachment of polyethylene glycol ("PEG") polymers, polyamino acids (e.g., poly-his, poly-arg, poly-lys, etc.) and/or fatty acid chains of various lengths to the N- or C-terminus or amino acid residue side chains of an ORP analog. Small molecule substituents include short alkyls and constrained alkyls (*e.g.*, branched, cyclic, fused, adamantyl), and aromatic groups.

By "ORP agonist" is meant a compound which elicits a biological activity of one of the polypeptide sequences provided in SEQ ID NOs: 1 through 27. In a preferred embodiment, the term "ORP agonist" refers to a compound which elicits a biological effect in the reduction of food intake similar to that of amylin, for example a compound (1) having activity in the food intake, gastric emptying, pancreatic secretion, or weight loss assays similar to an ORP of the present invention, and (2) which binds in an amylin receptor binding assay as an ORP of the present invention. Preferably, ORP agonists will bind in such assays with an affinity of greater than 1 μM, and more preferably with an affinity of greater than 1-10 nM. Such agonists may comprise a polypeptide having a functional ORP domain, an active fragment of an ORP, or a small chemical molecule.

ORP agonists may be peptide or non-peptide compounds, and include "ORP agonist analog polypeptides," which refer to any ORP analog polypeptides that have food intake lowering activity, typically by virtue of binding to or otherwise directly or indirectly interacting with an amylin receptor or other receptor or receptors with which an ORP itself may interact to elicit a biological response. One skilled in the art will appreciate that the "ORP agonists" as described above and the "ORP analogs" of the present invention are two different concepts with different scopes, although their coverage may sometimes overlap.

By "amino acid" and "amino acid residue" is meant natural amino acids, unnatural amino acids, and modified amino acid, all in their D and L stereoisomers if their structure allow such stereoisomeric forms. Natural amino acids include alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine(Ile), leucine (Leu), Lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), typtophan (Trp), tyrosine (Tyr) and valine (Val). Unnatural amino acids include, but are not limited to azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2aminoisobutyric acid, 3-aminoisbutyric acid, 2-aminopimelic acid, tertiary-butylglycine, 2,4-diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, homoproline, hydroxylysine, allo-hydroxylysine, 3-hydroxyproline, 4-hydroxyproline, isodesmosine, allo-isoleucine, N-methylalanine, N-methylglycine, N-methylisoleucine, N-methylpentylglycine, N-methylvaline, naphthalanine, norvaline, norleucine, ornithine, pentylglycine, pipecolic acid and thioproline. Modified amino acid include the natural and unnatural amino acids which are chemically blocked, reversibly or irreversibly, or modified on their N-terminal amino group or their side chain groups, as for example, methionine sulfoxide, methionine sulfone, S (carbo amino group or side chain functional group has been chemically codified to another functional group. For example, asparticacid- (beta-methyl ester) is amodified amino acid of aspartic acid; N-ethylglycine is a modified amino acid of glycine; or alanine carboxamide is a modified amino acid of alanine. Additional residues that can be incorporated are described by

Sandberg *et. al.* (Sandberg, M., Eriksson, L., Sjostrom, M., and Wold, S. New chemical descriptors relevant for the design of biologically active peptides. A multivariate characterization of 87 amino acids. J. Med. Chem. (1998) 41, 2481-2491.)

As discussed above, in one aspect, the present invention relates to ORPs including one or more amino acid sequence modifications. Such modifications include substitutions, insertions, and/or deletions, alone or in combination. In a preferred aspect, the ORPs of the invention include one or more modifications of a "non-essential" amino acid residue. In the context of the invention, a "non-essential" amino acid residue is a residue that can be altered, i.e., deleted or substituted, in the ORP amino acid sequence without abolishing or substantially reducing the ORP agonist activity of the analog polypeptide. Preferably, the ORP analog polypeptides of the invention retain at least about 25%, preferably about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% percent of the biological activity of an ORP with regard to the reduction of food intake. In another embodiment, the analog polypeptides of the invention exhibit improved ORP activity. Preferably, the analog polypeptides of the invention exhibits at least about 110%, 125%, 130%, 140%, 150%, 200%, or more of the biological activity of ORPs with regard to the reduction of food intake.

Preferred analog polypeptide are those having a potency in one of the assays described herein (preferably food intake or weight reduction assays) which is greater than the potency of amylin in that same assay.

Preferred substitutions include conserved amino acid substitutions. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain, or physicochemical characteristics (*e.g.*, electrostatic, hydrogen bonding, isosteric, hydrophobic features). Families of amino acid residues having similar side chains are known in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, methionine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan), β-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

By "condition or disorder which can be alleviated by reducing caloric (or nutrient) intake or availability" is meant any condition or disorder in a subject that is either caused by, complicated by, or aggravated by a relatively high nutrient intake or availability, or that can be alleviated by reducing nutrient intake or availability, for example by decreasing food intake. Such conditions or disorders include, but are not limited to, obesity, diabetes mellitus, including type 2 diabetes, eating disorders, and insulin-resistance syndromes.

Thus, disorders include, but are not limited to, obesity, anorexia, cachexia, bulimia, and other wasting diseases characterized by loss of appetite, diminished food intake, or body weight loss. Complications include, but are not limited to, insulin resistance, diabetes mellitus, hypertension, cardiovascular disease, pseudotumor, cerebri, hyperlipidemia, sleep apnea, cancer, pulmonary hypertension, cardiovascular disease, cholecystitis, and osteoarthritis.

While "obesity" is generally defined as a body mass index over 30, for purposes of this disclosure, any subject, including those with a body mass index of less than 30, who needs or wishes to reduce body weight is included in the scope of "obese."

As used herein, the phrase "pharmaceutically acceptable" refers to an agent that does not interfere with the effectiveness of the biological activity of an active ingredient, and which may be approved by a regulatory agency of the Federal government or a state government, or is listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly for use in humans. Accordingly, suitable pharmaceutically acceptable carriers include agents that do not interfere with the effectiveness of a pharmaceutical composition.

As used herein, the phrase "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable, preferably nontoxic, acids and bases, including inorganic and organic acids and bases, including but not limited to, sulfuric, citric, maleic, acetic, oxalic, hydrochloride, hydro bromide, hydro iodide, nitrate, sulfate, bisulfite, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and pamoate

(i.e., 1,1'-methylene-bis-(2-hydroxy-3-nap- hthoate)) salts. Pharmaceutically acceptable salts include those formed with free amino groups such as, but not limited to, those derived from hydrochloric, phosphoric, acetic, oxalic, and tartaric acids. Pharmaceutically acceptable salts also include those formed with free carboxyl groups such as, but not limited to, those derived from sodium, potassium, ammonium, sodium lithium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, and procaine.

As used herein, the phrase "isolated polypeptide or peptide" refers to a polypeptide or peptide that is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the protein, peptide, or fragment thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, i.e., it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly such preparations of the protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the polypeptide of interest. In preferred embodiments, purified or isolated preparations will lack any contaminating proteins from the same animal from which the protein is normally produced, as can be accomplished by recombinant expression of, for example, a human protein in a non-human cell.

The polypeptides or analogs thereof of the present invention can also be used in diagnostic applications for determining conditions or disorders associated with caloric intake.

The polypeptides of the present invention for use in the methods of the present invention demonstrate affinity to receptors in the amylin/CGRP/calcitonin family. In particular, the polypeptides or analogs thereof of the present invention show a significant affinity for binding to the amylin receptor, as well as the ability to bind to other receptors such as calcitonin and CGRP receptors. Preferably, the polypeptides of the present invention bind an amylin receptor with an affinity of greater than 20nM, 10 nM, 5 nM, 1nM, and more preferably with an affinity of greater than 0.10 nM.

The polypeptides of the present invention can also bind to the calcitonin and CGRP receptors, preferably with lower affinity than for the amylin receptor. The polypeptides preferably bind to a calcitonin receptor with an affinity of greater than 500 nM, 250 nM, 100 nM and to a CGRP receptor with an affinity of greater than about 1 μM, 700 nM, 500 nM.

The polypeptides of the present invention also preferably demonstrate activity in food intake assays. Preferably, the polypeptides or analogs thereof demonstrate greater activity in food intake assays than that of amylin.

The polypeptides demonstrate the ability to reduce cumulative food intake more than 5% over administration of the vehicle, preferably more than 15%, more preferably more than 25%, even more preferably more than 35%, most preferably more than 50% over the vehicle.

The polypeptides of the present invention also show the ability to reduce the body weight of an obese subject.

Preparation of Obesity Related Polypeptides

The ORPs described herein may be prepared using standard recombinant techniques or chemical peptide synthesis techniques known in the art, e.g., using an automated or semi-automated peptide synthesizer, or both. Likewise, the derivatives of the polypeptides of the invention may be produced using standard chemical, biochemical, or in vivo methodologies.

The ORPs of the invention can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. *See, e.g.*,

Stewart and Young, Solid Phase Peptide Synthesis, 2d. *ed.*, Pierce Chemical Co. (1984); Tam *et al.*, J. Am. Chem. Soc. 105: 6442 (1983); Merrifield, Science 232: 341-7 (1986); and Barany and Merrifield, The Peptides, Gross and Meienhofer, eds., Academic Press, New York, 1-284 (1979). Solid phase peptide synthesis may be carried out with an automatic peptide synthesizer (*e.g.*, Model 430A, Applied Biosystems Inc., Foster City, California) using the NMP/HOBt (Option 1) system and tBoc or Fmoc chemistry (*see*, Applied Biosystems User's Manual for the ABI 430A Peptide Synthesizer, Version 1.3B July 1, 1988, section 6, pp. 49-70, Applied Biosystems, Inc., Foster City, California) with capping. Peptides may also be assembled using an Advanced Chem Tech Synthesizer (Model MPS 350, Louisville, Kentucky). Peptides may be purified by RP-HPLC (preparative and analytical) using, e.g., a Waters Delta Prep 3000 system and a C4, C8, or C18 preparative column (10 μ, 2.2x25 cm; Vydac, Hesperia, California). The active protein can be readily synthesized and then screened in screening assays designed to identify reactive peptides.

The ORPs of the present invention may alternatively be produced by recombinant techniques well known in the art. *See, e.g.*, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d *ed.*, Cold Spring Harbor (1989). These polypeptides produced by recombinant technologies may be expressed from a polynucleotide. These polynucleotide sequences may incorporate codons facilitating transcription and translation of mRNA in microbial hosts. Such manufacturing sequences may readily be constructed according to the methods well known in the art. *See, e.g.*, WO 83/04053. The polynucleotides above may also optionally encode an N-terminal methionyl residue. Non-peptide compounds useful in the present invention may be prepared by art-known methods. For example, phosphate-containing amino acids and peptides containing such amino acids may be prepared using methods known in the art. *See, e.g.*, Bartlett and Landen, Bioorg. Chem. 14: 356-77 (1986).

A variety of expression vector/host systems may be utilized to contain and express an ORP coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transfected

with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmid); or animal cell systems. Mammalian cells that are useful in recombinant protein productions include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, COS cells (such as COS-7), WI 38, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562 and 293 cells. Exemplary protocols for the recombinant expression of the protein are described herein below.

As such, polynucleotide sequences provided by the invention are useful in generating new and useful viral and plasmid DNA vectors, new and useful transformed and transfected procaryotic and eucaryotic host cells (including bacterial, yeast, and mammalian cells grown in culture), and new and useful methods for cultured growth of such host cells capable of expression of the present polypeptides. The polynucleotide sequences encoding ORPs herein may also be useful for gene therapy.

The present invention also provides for processes for recombinant DNA production of the present ORPs. Provided is a process for producing the polypeptides from a host cell containing nucleic acids encoding such ORPs comprising: (a) culturing said host cell containing polynucleotides encoding such polypeptides under conditions facilitating the expression of such DNA molecule; and (b) obtaining such ORPs.

Host cells may be prokaryotic or eukaryotic and include bacteria, mammalian cells (such as Chinese Hamster Ovary (CHO) cells, monkey cells, baby hamster kidney cells, cancer cells or other cells), yeast cells, and insect cells.

Mammalian host systems for the expression of the recombinant protein also are well known to those of skill in the art. Host cell strains may be chosen for a particular ability to process the expressed protein or produce certain post-translation modifications that will be useful in providing protein activity. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing, which cleaves a "prepro" form of the protein, may also be important for correct insertion, folding and/or function. Different host cells, such as CHO, HeLa, MDCK, 293, WI38, and the like, have specific cellular machinery and characteristic mechanisms for such post-translational activities,

and may be chosen to ensure the correct modification and processing of the introduced foreign protein.

Alternatively, a yeast system may be employed to generate the ORPs of the present invention. The coding region of an ORP DNA is amplified by PCR. A DNA encoding the yeast pre-pro-alpha leader sequence is amplified from yeast genomic DNA in a PCR reaction using one primer containing nucleotides 1-20 of the alpha mating factor gene and another primer complementary to nucleotides 255-235 of this gene (Kurjan and Herskowitz, Cell, 30: 933-43 (1982)). The pre-pro-alpha leader coding sequence and ORP coding sequence fragments are ligated into a plasmid containing the yeast alcohol dehydrogenase (ADH2) promoter, such that the promoter directs expression of a fusion protein consisting of the pre-pro-alpha factor fused to the mature ORP. As taught by Rose and Broach, Meth. Enz. 185: 234-79, Goeddel ed., Academic Press, Inc., San Diego, California (1990), the vector further includes an ADH2 transcription terminator downstream of the cloning site, the yeast "2-micron" replication origin, the yeast leu-2d gene, the yeast REP1 and REP2 genes, the E. coli β-lactamase gene, and an E. coli origin of replication. The β-lactamase and leu-2d genes provide for selection in bacteria and yeast, respectively. The leu-2d gene also facilitates increased copy number of the plasmid in yeast to induce higher levels of expression. The REP1 and REP2 genes encode proteins involved in regulation of the plasmid copy number.

The DNA construct described in the preceding paragraph is transformed into yeast cells using a known method, e.g., lithium acetate treatment (Steams *et al.*, Meth. Enz. 185: 280-97 (1990)). The ADH2 promoter is induced upon exhaustion of glucose in the growth media (Price *et al.*, Gene 55: 287 (1987)). The pre-pro-alpha sequence effects secretion of the fusion protein from the cells. Concomitantly, the yeast KEX2 protein cleaves the pre-pro sequence from the mature ORPs (Bitter *et al.*, Proc. Natl. Acad. Sci. USA 81: 5330-4 (1984)).

The ORPs may also be recombinantly expressed in yeast using a commercially available expression system, *e.g.*, the Pichia Expression System (Invitrogen, San Diego, California), following the manufacturer's instructions. This system also relies on the pre-pro-alpha sequence to direct secretion, but transcription of the insert is driven by the alcohol oxidase (AOX1) promoter upon induction by methanol. The secreted ORP is

purified from the yeast growth medium by, *e.g.*, the methods used to purify ORP from bacterial and mammalian cell supernatants.

Alternatively, the DNA encoding ORPs may be cloned into the baculovirus expression vector pVL1393 (PharMingen, San Diego, California). This ORPs-containing vector is then used according to the manufacturer's directions (PharMingen) to infect Spodoptera frugiperda cells in sF9 protein-free media and to produce recombinant protein. The protein is purified and concentrated from the media using a heparin-Sepharose column (Pharmacia, Piscataway, New Jersey) and sequential molecular sizing columns (Amicon, Beverly, Massachusetts), and resuspended in PBS. SDS-PAGE analysis shows a single band and confirms the size of the protein, and Edman sequencing on a Proton 2090 Peptide Sequencer confirms its N-terminal sequence.

For example, the DNA sequence encoding the desired ORP may be cloned into a plasmid containing a desired promoter and, optionally, a leader sequence (*see, e.g.*, Better et al., Science 240: 1041-3 (1988)). The sequence of this construct may be confirmed by automated sequencing. The plasmid is then transformed into E. coli, strain MC1061, using standard procedures employing CaCl2 incubation and heat shock treatment of the bacteria (Sambrook *et al.*, *supra*). The transformed bacteria are grown in LB medium supplemented with carbenicillin, and production of the expressed protein is induced by growth in a suitable medium. If present, the leader sequence will affect secretion of the mature ORP and be cleaved during secretion. The secreted recombinant protein is purified from the bacterial culture media by the method described herein below.

Alternatively, the polypeptides of the present invention may be expressed in an insect system. Insect systems for protein expression are well known to those of skill in the art. In one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The ORP coding sequence is cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of ORP will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect S. frugiperda cells or Trichoplusia larvae in which the polypeptide is

expressed (Smith *et al.*, J. Virol. 46: 584 (1983); Engelhard *et al.*, Proc. Natl. Acad. Sci. USA 91: 3224-7 (1994)).

In another example, the DNA sequence encoding the ORP may be amplified by PCR and cloned into an appropriate vector, for example, pGEX-3X (Pharmacia, Piscataway, New Jersey). The pGEX vector is designed to produce a fusion protein comprising glutathione-S-transferase (GST), encoded by the vector, and a protein encoded by a DNA fragment inserted into the vector's cloning site. The primers for the PCR may be generated to include, for example, an appropriate cleavage site. The recombinant fusion protein may then be cleaved from the GST portion of the fusion protein. The pGEX-3X/PYY analog polypeptide construct is transformed into E. coli XL-1 Blue cells (Stratagene, La Jolla, California), and individual transformants are isolated and grown at 37°C in LB medium (supplemented with carbenicillin) to an optical density at wavelength 600 nm of 0.4, followed by further incubation for 4 hours in the presence of 0.5 mM Isopropyl β-D-Thiogalactopyranoside (Sigma Chemical Co., St. Louis, Missouri). Plasmid DNA from individual transformants is purified and partially sequenced using an automated sequencer to confirm the presence of the desired ORP-encoding gene insert in the proper orientation.

The fusion protein, expected to be produced as an insoluble inclusion body in the bacteria, may be purified as follows. Cells are harvested by centrifugation; washed in 0.15 M NaCl, 10 mM Tris, pH 8, 1 mM EDTA; and treated with 0.1 mg/mL lysozyme (Sigma Chemical Co.) for 15 min. at room temperature. The lysate is cleared by sonication, and cell debris is pelleted by centrifugation for 10 min. at 12,000xg. The fusion protein-containing pellet is resuspended in 50 mM Tris, pH 8, and 10 mM EDTA, layered over 50% glycerol, and centrifuged for 30 min. at 6000xg. The pellet is resuspended in standard phosphate buffered saline solution (PBS) free of Mg^{++} and Ca^{++}. The fusion protein is further purified by fractionating the resuspended pellet in a denaturing SDS polyacrylamide gel (Sambrook et al., supra). The gel is soaked in 0.4 M KCl to visualize the protein, which is excised and electroeluted in gel-running buffer lacking SDS. If the GST/ ORP fusion protein is produced in bacteria as a soluble protein, it may be purified using the GST Purification Module (Pharmacia Biotech).

The fusion protein may be subjected to digestion to cleave the GST from the mature ORP. The digestion reaction (20-40 μg fusion protein, 20-30 units human thrombin (4000 U/mg (Sigma) in 0.5 mL PBS) is incubated 16-48 hrs. at room temperature and loaded on a denaturing SDS-PAGE gel to fractionate the reaction products. The gel is soaked in 0.4 M KCl to visualize the protein bands. The identity of the protein band corresponding to the expected molecular weight of the ORP may be confirmed by partial amino acid sequence analysis using an automated sequencer (Applied Biosystems Model 473A, Foster City, California).

In a particularly preferred method of recombinant expression of the ORPs of the present invention, 293 cells may be co-transfected with plasmids containing the ORP DNA in the pCMV vector (5' CMV promoter, 3' HGH poly A sequence) and pSV2neo (containing the neo resistance gene) by the calcium phosphate method. Preferably, the vectors should be linearized with ScaI prior to transfection. Similarly, an alternative construct using a similar pCMV vector with the neo gene incorporated can be used. Stable cell lines are selected from single cell clones by limiting dilution in growth media containing 0.5 mg/mL G418 (neomycin-like antibiotic) for 10-14 days. Cell lines are screened for ORP expression by ELISA or Western blot, and high-expressing cell lines are expanded for large scale growth.

It is preferable that the transformed cells are used for long-term, high-yield protein production and as such stable expression is desirable. Once such cells are transformed with vectors that contain selectable markers along with the desired expression cassette, the cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The selectable marker is designed to confer resistance to selection, and its presence allows growth and recovery of cells that successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell.

A number of selection systems may be used to recover the cells that have been transformed for recombinant protein production. Such selection systems include, but are not limited to, HSV thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase genes, in tk-, hgprt- or aprt- cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for dhfr, that confers

resistance to methotrexate; gpt, that confers resistance to mycophenolic acid; neo, that confers resistance to the aminoglycoside, G418; also, that confers resistance to chlorsulfuron; and hygro, that confers resistance to hygromycin. Additional selectable genes that may be useful include trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine. Markers that give a visual indication for identification of transformants include anthocyanins, β-glucuronidase and its substrate, GUS, and luciferase and its substrate, luciferin.

Many of the ORPs of the present invention may be produced using a combination of both automated peptide synthesis and recombinant techniques. For example, an ORP of the present invention may contain a combination of modifications including deletion, substitution, and insertion by PEGylation. Such an ORP may be produced in stages. In the first stage, an intermediate ORP containing the modifications of deletion, substitution, insertion, and any combination thereof, may be produced by recombinant techniques as described. Then after an optional purification step as described below, the intermediate polypeptide is PEGylated through chemical modification with an appropriate PEGylating reagent (*e.g.*, from Nectar Transforming Therapeutics, San Carlos, California) to yield the desired ORP. One skilled in the art will appreciate that the above-described procedure may be generalized to apply to a ORP containing a combination of modifications selected from deletion, substitution, insertion, derivation, and other means of modification well known in the art and contemplated by the present invention.

It may be desirable to purify the ORPs generated by the present invention. Peptide purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography, polyacrylamide gel electrophoresis, and isoelectric focusing. A particularly efficient method of purifying peptides is reverse phase HPLC, followed by characterization of purified product by liquid chromatography/mass spectrometry (LC/MS) and Matrix-Assisted Laser

Desorption Ionization (MALDI) mass spectrometry. Additional confirmation of purity is obtained by determining amino acid analysis.

Certain aspects of the present invention concern the purification, and in particular embodiments, the substantial purification, of an encoded protein or peptide. The term "purified peptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the peptide is purified to any degree relative to its naturally obtainable state. A purified peptide therefore also refers to a peptide, free from the environment in which it may naturally occur.

Generally, "purified" will refer to a peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a composition in which the peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the peptides in the composition.

Various techniques suitable for use in peptide purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies, and the like; heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

There is no general requirement that the peptides always be provided in their most purified state. Indeed, it is contemplated that less substantially purified products will have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed, utilizing an HPLC apparatus, will generally result in a greater "-fold" purification than the same technique utilizing a low pressure

chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of protein product, or in maintaining the activity of an expressed protein.

One may optionally purify and isolate such ORPs from other components obtained in the process. Methods for purifying a polypeptide can be found in U.S. Patent No. 5,849,883. These documents describe specific exemplary methods for the isolation and purification of G-CSF compositions that may be useful in isolating and purifying the ORPs of the present invention. Given the disclosure of these patents, it is evident that one of skill in the art would be well aware of numerous purification techniques that may be used to purify polypeptides from a given source.

Also it is contemplated that a combination of anion exchange and immunoaffinity chromatography may be employed to produce purified ORP compositions of the present invention.

Certain preferred methods for synthesis are described in the commonly-assigned patent application Serial No. 454,533 (file December 6, 1999) the entirety of which is incorporated herein by reference.

Use of ORPs in the Treatment of Metabolic Conditions or Disorders

In another aspect of the present invention, the ORPs are used in methods for the prevention or treatment of conditions or disorders associated with reducing caloric intake. The disorders or conditions prevented or treated include, but are not limited to obesity, anorexia, bulimia, cachexia, insulin resistance, diabetes mellitus, hypertension, cardiovascular disease, and gastric dumping. The polypeptide compounds of the present invention may also be used in methods of controlling appetite in a subject.

Binding assays for determining interactions with the amylin-receptor, the calcitonin-receptor, and the CGRP receptor are described for example in U.S. Patent No. 5,264,372, the entirety of which is incorporated herein by reference.

The polypeptides described herein are useful in view of their pharmacological properties. In particular, the compounds of the invention possess activity as agents to reduce caloric intake. They can be used to treat conditions or diseases which can be alleviated by reducing caloric intake.

As such, in another aspect of the invention, methods for treating or preventing obesity are provided, wherein the method comprises administering a therapeutically or prophylactically effective amount of an ORP to a subject in need thereof. In a preferred embodiment, the subject is an obese or overweight subject. As described above, while "obesity" is generally defined as a body mass index over 30, for purposes of this disclosure, any subject, including those with a body mass index of less than 30, who needs or wishes to reduce body weight is included in the scope of "obese." Subjects who are insulin resistant, glucose intolerant, or have any form of diabetes mellitus (e.g., type 1, 2 or gestational diabetes) can benefit from this method.

In another aspect of the present invention, methods for reducing body weight of a subject are provided, wherein the methods comprise administering to a subject an effective amount of an ORP of the present invention. In a preferred embodiment, the methods of the invention are used to reduce the body weight of an obese subject, including a subject a subject desirous of reducing body weight.

In other aspects of the invention, methods of reducing food intake, reducing nutrient availability, causing weight loss, treating diabetes mellitus, and improving lipid profile (including reducing LDL cholesterol and triglyceride levels and/or changing HDL cholesterol levels) are provided, wherein the methods comprise administering to a subject an effective amount of a polypeptide of the present invention. In a preferred embodiment, the methods of the invention are used to treat or prevent conditions or disorders which can be alleviated by reducing nutrient availability in a subject in need thereof, comprising administering to said subject a therapeutically or prophylactically effective amount of a polypeptide of the present invention. Such conditions and disorders include, but are not limited to, hypertension, dyslipidemia, cardiovascular disease, eating disorders, insulin-resistance, obesity, and diabetes mellitus of any kind.

In the methods of the invention, preferred polypeptides are those having a potency in one of the assays described herein (preferably food intake or weight reduction assays) which is greater than the potency of amylin in that same assay.

For all indications, in preferred embodiments, the ORPs are administered peripherally at a dose of about 1 μg to about 5 mg per day in single or divided doses or controlled continual release, or at about 0.01 μg/kg to about 500 μg/kg per dose, more

preferably about 0.05 μg/kg to about 250 μg/kg, most preferably below about 50 μg/kg. Dosages in these ranges will vary with the potency of each analog or derivative, of course, and may be determined by one of skill in the art.

In the methods of the present invention, the polypeptides may be administered separately or together with one or more other compounds and compositions that exhibit a long term or short-term action to reduce nutrient availability, including, but not limited to other compounds and compositions that comprise an amylin or amylin analog agonist, salmon calcitonin, a cholecystokinin (CCK) or CCK agonist, a leptin (OB protein) or leptin agonist, an exendin or exendin analog agonist, or a GLP-1 or GLP-1 analog agonist or a PYY or PYY analog, or a PYY related polypeptide. Suitable amylin agonists include, for example, [25,28,29 Pro-]-human amylin (also known as "pramlintide," and described in U.S. Pat. Nos. 5,686,511 and 5,998,367). The CCK used is preferably CCK octopeptide (CCK-8). Leptin is discussed in, for example, (Pelleymounter, Cullen et al., Science 269: 540-543 (1995); Halaas, Gajiwala et al., Science 269: 543-6 (1995); Campfield, Smith et al., Science 269: 546-549 (1995)). Suitable exendins include exendin-3 and exendin-4, and exendin agonist compounds include, for example, those described in PCT Publications WO 99/07404, WO 99/25727, and WO 99/25728. Suitable PYY polypeptides and analogs include those described in U.S. Application No.: [Attorney Docket Nos. 18528.662 and 18528.663].

In the methods of the present invention the body weight of an obese subject is preferably reduced.

Pharmaceutical Compositions

The present invention also relates to pharmaceutical compositions comprising a therapeutically or prophylactically effective amount of at least one ORP of the invention, or a pharmaceutically acceptable salt thereof, together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in the delivery of the ORPs. Such compositions may include diluents of various buffer content (*e.g.*, Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (*e.g.*, Tween 80, Polysorbate 80), anti-oxidants (*e.g.*, ascorbic acid, sodium metabisulfite), preservatives (*e.g.*, thimersol, benzyl alcohol), and bulking

substances (*e.g.*, lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds, such as polylactic acid, polyglycolic acid, etc., or in association with liposomes. Such compositions will influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present ORPs. *See, e.g.*, Remington's Pharmaceutical Sciences 1435-712, 18th *ed.*, Mack Publishing Co., Easton, Pennsylvania (1990).

In general, the present ORPs will be useful in the same way that amylin is useful in view of their pharmacological properties. One preferred use is to peripherally administer such ORPs for the treatment or prevention of metabolic conditions and disorders. In particular, the compounds of the invention possess activity as agents to reduce nutrient availability, reduce food intake, and effect weight loss.

The present ORPs may be formulated for peripheral administration, including formulation for injection, oral administration, nasal administration, pulmonary administration, topical administration, or other types of administration as one skilled in the art will recognize. More particularly, administration of the pharmaceutical compositions according to the present invention may be via any common route so long as the target tissue is available via that route. In a preferred embodiment, the pharmaceutical compositions may be introduced into the subject by any conventional peripheral method, *e.g.*, by intravenous, intradermal, intramusclar, intramammary, intraperitoneal, intrathecal, retrobulbar, intrapulmonary (*e.g.*, term release); by oral, sublingual, nasal, anal, vaginal, or transdermal delivery, or by surgical implantation at a particular site. The treatment may consist of a single dose or a plurality of doses over a period of time. Controlled continual release of the compositions of the present invention is also contemplated.

The formulation may be liquid or may be solid, such as lyophilized, for reconstitution. Aqueous compositions of the present invention comprise an effective amount of the ORP, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. The phrase "pharmaceutically or pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media,

coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions. In some cases, it will be convenient to provide an ORP and another food-intake-reducing, plasma glucose-lowering or plasma lipid-altering agent, such as an amylin, an amylin agonist analog, a CCK or CCK agonist, or a leptin or leptin agonist, or an exendin or exendin agonist analog, or a PYY or a PYY analog, in a single composition or solution for administration together. In other cases, it may be more advantageous to administer the additional agent separately from said ORP.

The ORP of the invention may be prepared for administration as solutions of free base, or pharmacologically acceptable salts in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups also can be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Such products are readily prepared by procedures well known to those skilled in the art. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

In one embodiment, the pharmaceutical compositions of the present invention are formulated so as to be suitable for parenteral administration, *e.g.*, via injection or infusion. Preferably, the ORP is suspended in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 3.0 to about 8.0, preferably at a pH of about 3.5 to about 7.4, 3.5 to 6.0, or 3.5 to about 5.0. Useful buffers include sodium citrate-citric acid and sodium phosphate-phosphoric acid, and sodium acetate/acetic acid buffers. A form of repository or "depot" slow release preparation may be used so that

therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or delivery.

The pharmaceutical compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form should be sterile and should be fluid to the extent that easy syringability exists. It is also desirable for the ORP of the invention to be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial an antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents (for example, sugars or sodium chloride). Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption (for example, aluminum monostearate and gelatin).

Sterile injectable solutions may be prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle that contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Generally, a therapeutically or prophylactically effective amount of the present ORPs will be determined by the age, weight, and condition or severity of the diseases or metabolic conditions or disorders of the recipient. *See, e.g.,* Remington's Pharmaceutical

Sciences 697-773. *See also* Wang and Hanson, Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers, Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S (1988). Typically, a dosage of between about 0.001 μg/kg body weight/day to about 1000 μg/kg body weight/day, may be used, but more or less, as a skilled practitioner will recognize, may be used. Dosing may be one or more times daily, or less frequently, and may be in conjunction with other compositions as described herein. It should be noted that the present invention is not limited to the dosages recited herein.

Appropriate dosages may be ascertained through the use of established assays for determining level of metabolic conditions or disorders in conjunction with relevant dose-response data. The final dosage regimen will be determined by the attending physician, considering factors that modify the action of drugs, e.g., the drug's specific activity, severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding appropriate dosage levels and duration of treatment for specific diseases and conditions.

An effective dose will typically be in the range of about 1 to 30 μg to about 5 mg/day, preferably about 10 to 30 μg to about 2 mg/day and more preferably about 5 to 100 μg to about 1 mg/day, most preferably about 5 μg to about 500 μg/day, for a 50 kg patient, administered in a single or divided doses. Preferably, dosages are between about 0.01 to about 100 μg/kg/dose. The exact dose to be administered may be determined by one of skill in the art and is dependent upon the potency of the particular compound, as well as upon the age, weight and condition of the individual. Administration should begin whenever the suppression of nutrient availability, food intake, weight, blood glucose or plasma lipid lowering is desired, for example, at the first sign of symptoms or shortly after diagnosis of obesity, diabetes mellitus, or insulin-resistance syndrome. Administration may be by any route, *e.g.*, injection, preferably subcutaneous or intramuscular, oral, nasal, transdermal, etc. Dosages for certain routes, for example oral administration, may be increased to account for decreased bioavailablity, for example, by about 5-100 fold.

In one embodiment, where the pharmaceutical formulation is to be administered parenterally, the composition is formulation so as to deliver a dose of ORP ranging from 1 μg/kg to 100 mg/kg body weight/day, preferably at doses ranging from 0.1 mg/kg to about 50 mg/kg body weight/day. Parenteral administration may be carried out with an initial bolus followed by continuous infusion to maintain therapeutic circulating levels of drug product. Those of ordinary skill in the art will readily optimize effective dosages and administration regimens as determined by good medical practice and the clinical condition of the individual patient.

The frequency of dosing will depend on the pharmacokinetic parameters of the agents and the routes of administration. The optimal pharmaceutical formulation will be determined by one of skill in the art depending on the route of administration and the desired dosage. *See, e.g.*, Remington's Pharmaceutical Sciences, *supra*, pages 1435-1712. Such formulations may influence the physical state, stability, rate of in vivo release and rate of in vivo clearance of the administered agents. Depending on the route of administration, a suitable dose may be calculated according to body weight, body surface areas or organ size. Further refinement of the calculations necessary to determine the appropriate treatment dose is routinely made by those of ordinary skill in the art without undue experimentation, especially in light of the dosage information and assays disclosed herein, as well as the pharmacokinetic data observed in animals or human clinical trials.

It will be appreciated that the pharmaceutical compositions and treatment methods of the invention may be useful in fields of human medicine and veterinary medicine. Thus the subject to be treated may be a mammal, preferably human or other animal. For veterinary purposes, subjects include for example, farm animals including cows, sheep, pigs, horses and goats, companion animals such as dogs and cats, exotic and/or zoo animals, laboratory animals including mice, rats, rabbits, guinea pigs and hamsters; and poultry such as chickens, turkeys, ducks and geese.

In addition, the present invention contemplates a kit comprising an ORP of the invention, components suitable for preparing said ORP for pharmaceutical application, and instructions for using said ORP and components for pharmaceutical application.

To assist in understanding the present invention, the following Examples are included. The experiments relating to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

EXAMPLES

Example 1: Synthesis of the Caloric Intake Lowering Polypeptides

The following polypeptides can be synthesized using standard polypeptide synthesis methods. Such methods are described below and in USPN 6,610,824 and USPN 5,686,411 and in patent application Serial No. 454,533 (filed December 6, 1999), the entireties of which are incorporated herein by reference.

The polypeptides are assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.). In general, single-coupling cycles are used throughout the synthesis and Fast Moc (HBTU activation) chemistry is employed. However, at some positions coupling may be less efficient than expected and double couplings required. Deprotection (Fmoc group removal) of the growing peptide chain using piperidine likewise may not always be efficient and require double deprotection. Final deprotection of the completed peptide resin is achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptides are precipitated in ether/water (50 mL) and centrifuged. The precipitate is reconstituted in glacial acetic acid and lyophilized. The lyophilized peptides are dissolved in water). Crude purity is then determined.

Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN) are used in purification and analysis steps.

Solutions containing the various polypeptides are applied to a preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions are determined isocratically using a C-18 analytical column. Pure fractions are pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide to determine retention time.

The following 27 polypeptides were synthesized and tested in food intake assays:

c(KCNTATCATQRLANFLVRSSNNLTNVGSNTY-NH2) (SEQ ID NO: 1),
c(KCNTATCATQRLANELVRLQTYPRTNVGSNTY-NH2) (SEQ ID NO: 2),
c(CSNLSTCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 3),
c(KCNTATCVLGRLSQELHRLQTYPRTNTGSNTY)-NH2) (SEQ ID NO: 4),
Isocap-STAVL-(Aib)-K(formyl)-LSQEL-(Aib)-K(formyl)-LQTYPRTNTGSGTP-NH2 (SEQ ID NO: 5),
c(KCNTATCATQRLANALVHSSNNFGAILPSTNVGSNTY-NH2) (SEQ ID NO: 6),
c(KCNTATCATARLAAFLARSSGY-NH2) (SEQ ID NO: 7),
c(KCNTATCATQRLANFLVHSGNNFGAILSSTNVGSNTY-NH2) (SEQ ID NO: 8),
c(CNTATCATARLAAFLARS-NH2) (SEQ ID NO: 9),
c(KCNTATCVLGKLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 10),
c(KCNTATCVLGRLSQELHRLQTLPRTNTGSNTY-NH2) (SEQ ID NO: 11),
c(KCNTATCVLGRLSQELHRLQTYPPTNTGSNTY-NH2) (SEQ ID NO: 12),
c(KCNTATCVLGRLSQELHRLQTYPRTNVGSNTY-NH2) (SEQ ID NO: 13),
c(KCNTATCVLGRLSQELHRLQTLPPTNVGSNTY-NH2) (SEQ ID NO: 14),
c(KCNTATCVLGRLANFLHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 15),
c(ACNTATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 16),
c(KCATATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 17),
c(KCNAATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 18),
c(KCNTAACVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 19),
c(CANLSTCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 20),
Isocap-STAVLGRLSQELHRLQTYPRTNTGSNTY-NH2 (SEQ ID NO: 21),
c(CSNASTCVLGRLSQELHRLQTYPRTNTGSNTY-NH2 (SEQ ID NO: 22),
c(CSNLATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 23),
c(CSNLSACVLGRLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 24),
c(KCNTATCVLGRLSQELHKLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 25),
c(KCNTATCVLGRLSQELHRLQTYPRTNTGSGTP-NH2) (SEQ ID NO: 26), and
c(KCNTATCATQRLSQELHRLQTYPRTNTGSGTP-NH2) (SEQ ID NO: 27).

Example 2: Receptor Binding Assays

Initially, polypeptides can be used in assays to determine binding ability to amylin, calictonin and CGRP receptors.

Evaluation of the binding of compounds of the invention to amylin receptors can be carried out as follows. ^{125}I-rat amylin (Bolton-Hunter labeled at the N-terminal lysine) is purchased from Amersham Corporation (Arlington Heights, Ill.). Unlabeled peptides are obtained from BACHEM Inc. (Torrance, Calif.) and Peninsula Laboratories (Belmont, Calif.).

Male Sprague-Dawley rats (200-250) grams are sacrificed by decapitation. Brains are removed to cold phosphate-buffered saline (PBS). From the ventral surface, cuts are made rostral to the hypothalamus, bounded laterally by the olfactory tracts and extending at a 45 angle medially from these tracts. This basal forebrain tissue, containing the nucleus accumbens and surrounding regions, is weighed and homogenized in ice-cold 20 mM HEPES buffer (20 mM HEPES acid, pH adjusted to 7.4 with NaOH at 23C). Membranes are washed three times in fresh buffer by centrifugation for 15 minutes at 48,000.times.g. The final membrane pellet is resuspended in 20 mM HEPES buffer containing 0.2 mM phenylmethylsulfonyl fluoride (PMSF).

To measure ^{125}I-amylin binding, membranes from 4 mg original wet weight of tissue are incubated with ^{125}I -amylin at 12-16 pM in 20 mM HEPES buffer containing 0.5 mg/ml bacitracin, 0.5 mg/ml bovine serum albumin, and 0.2 mM PMSF. Solutions are incubated for 60 minutes at 2C. Incubations are terminated by filtration through GF/B glass fiber filters (Whatman Inc., Clifton, N.J.) which has been presoaked for 4 hours in 0.3% poylethyleneimine in order to reduce nonspecific binding of radiolabeled peptides. Filters are washed immediately before filtration with 5 ml cold PBS, and immediately after filtration with 15 ml cold PBS. Filters are removed and radioactivity assessed in a gamma-counter at a counting efficiency of 77%. Competition curves are generated by measuring binding in the presence of 10^{-12} to 10^{-6} M unlabeled test compound and are analyzed by nonlinear regression using a 4-parameter logistic equation (Inplot program; GraphPAD Software, San Diego).

In this assay, purified human amylin binds to its receptor at a measured IC_{50} of about 50 pM. Results for test compounds of the invention are set forth in the table below, showing that each of the compounds has significant receptor binding activity.

	Peptide Sequence	Amylin (nM)	CT(C1a) (nM)	CGRP (nM)
SEQ ID NO: 6	c(KCNTATCATQRLANALVHSS NNFGAILPSTNVGSNTY-NH2)	1.18	87.7	151
SEQ ID NO: 8	c(KCNTATCATQRLANFLVHSG NNFGAILSSTNVGSNTY-NH2)	0.13	0.45	
SEQ ID NO: 3	c(CSNLSTCVLGRLSQELHRLQT YPRTNTGSNTY-NH2)	0.02		23.93
SEQ ID NO: 4	c(KCNTATCVLGRLSQELHRLQT YPRTNTGSNTY-NH2)	0.01		1
SEQ ID NO: 2	c(KCNTATCATQRLANELVRLQT YPRTNVGSNTY-NH2)	0.01		0.61
SEQ ID NO: 1	c(KCNTATCATQRLANFLVRSSN NLTNVGSNTY-NH2)	0.24		
SEQ ID NO: 9	c(CNTATCATARLAAFLARS-NH2)	6.90	0.181	
SEQ ID NO: 7	c(KCNTATCATARLAAFLARSSG Y-NH2)	1.20	0.05	704.5
SEQ ID NO: 5	Isocap-STAVL-(Aib)-K(formyl)-LSQEL-(Aib)-K(formyl)-LQTYPRTNTGSGTP-NH2	5.55	0.28	251.3

Example 3: Activity of Polypeptides on Food Intake

Female NIH/Swiss mice (8-12 weeks old) are group housed with a 12:12 hour light:dark cycle with lights on at 0600. Water and a standard pelleted mouse chow diet are available ad libitum, except as noted. Animals are fasted and housed individually starting at approximately 1500 hrs, 1 day prior to experiment. The morning of the experiment (approx. 0630 hrs), all animals are weighed and divided into experimental groups so as to give the most similar weight distribution between groups. In a typical study, n=10 for the control group and at least 5 for each treatment group.

At time=0 min, all animals are given an intraperitoneal injection of vehicle or polypeptide in a volume of 5 ml/kg and immediately given a pre-weighed amount (10-15g) of the standard chow. Food is removed and weighed at 30, 60, 120 and 180 minutes to determine the amount of food consumed (Morley, Flood et al., Am J Physiol 267: R178-R184, 1994).

Food intake is calculated by subtracting the weight of the food remaining after 30 minutes, 60 minutes, 120 minutes, and 180 minutes from the weight of the food provided initially at time=0. The effects of treatment on food intake are expressed as % change relative to control.

As can be seen in the table below, the polypeptides administered peripherally (intraperitoneal injection) significantly reduced food intake measured at each time point in overnight-fasted female NIH/SW mice. The data at time points 30, 60, 120, and 180 minutes represents the decrease in cumulative food intake compared to the vehicle.

	Peptide Sequence	**FOOD INTAKE**(min)			
		30	60	120	180
AC2607 (SEQ ID NO: 6)	c(KCNTATCATQRLANALVHSSNNFGAILPSTNVGSNTY-NH2)	-41	-35	-37	-32
AC3071 (SEQ ID NO: 8)	c(KCNTATCATQRLANFLVHSGNNFGAILSSTNVGSNTY-NH2)	-28	-31	-34	-32
AC2306 (SEQ ID NO: 3)	c(CSNLSTCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	-87	-86	-79	-73
AC2307 (SEQ ID NO: 4)	c(KCNTATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	-66	-63	-55	
AC2221 (SEQ ID NO: 2)	c(KCNTATCATQRLANELVRLQTYPRTNVGSNTY-NH2)	-72	-67	-58	-51
AC0062 (SEQ ID NO: 1)	c(KCNTATCATQRLANFLVRSSNNLTNVGSNTY-NH2)	-63	-44	-37	
AC3098 (SEQ ID NO: 9)	c(CNTATCATARLAAFLARS-NH2)	-32	--	--	--
AC3052 (SEQ ID NO: 7)	c(KCNTATCATARLAAFLARSSGY-NH2)	-26	--	--	--
AC2389	Isocap-STAVL-(Aib)-K(formyl)-LSQEL-(Aib)-	-19	-27	-31	-17

(SEQ ID NO: 5)	K(formyl)-LQTYPRTNTGSGTP-NH2				

Results of the Food Intake Assay demonstrate that the peptides reduced food intake in subjects. The most potent polypeptides are AC 0062 (SEQ ID NO: 1) , AC 2306 (SEQ ID NO: 3), AC 2607 (SEQ ID NO: 6) and AC 3071 (SEQ ID NO: 8). Of these compounds, AC 2306 is the most potent and has a more desirable sequence length (32 AA vs. 37 AA). AC 2307 (SEQ ID NO: 4), AC 2221 (SEQ ID NO: 2) and AC 2389 (SEQ ID NO: 5) are identified as additional leads and structure-activity-relationship analysis conducted.

The polypeptides synthesized are classified into two groups based on sequence homology to either amylin (from rat) or calcitonin (from salmon). The results are shown below for representative polypeptides.

Peptide Sequence	Homology
c(KCNTATCVLGKLSQELHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 10)	sCT like
c(KCNTATCVLGRLSQELHKLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 25)	sCT like
c(KCNTATCVLGRLSQELHRLQTYPRTNTGSGTP-NH2) (SEQ ID NO: 26)	sCT like
c(KCNTATCATQRLSQELHRLQTYPRTNTGSGTP-NH2) (SEQ ID NO: 27)	sCT like
c(KCNTATCVLGRLANFLHRLQTYPRTNTGSNTY-NH2) (SEQ ID NO: 15)	Amylin like
c(KCNTATCVLGRLSQELHRLQTLPRTNTGSNTY-NH2) (SEQ ID NO: 11)	Amylin like
c(KCNTATCVLGRLSQELHRLQTYPPTNTGSNTY-NH2) (SEQ ID NO: 12)	Amylin like
c(KCNTATCVLGRLSQELHRLQTYPRTNVGSNTY-NH2) (SEQ ID NO: 13)	Amylin like
c(KCNTATCVLGRLSQELHRLQTLPPTNVGSNTY-NH2) (SEQ ID NO: 14)	Amylin like

An attempt is also made to identify important residues at the N-terminus of the AC2307 peptide (SEQ ID NO: 4) by an alannie-scan (or Ala-scan). These peptides are shown below. Identification of essential vs. non-essential amino acids in the peptide can be achieved by preparing several peptides that have a shorter sequence than the full region (see 2 above) in which each amino acid is sequentially replaced by the amino acid Ala ("Ala-Scan."), or sequentially each amino acid is omitted ("omission-scan"). This allows the identification of amino acids which modulating activity is decreased by said replacement/omission ("essential") and which are not decreased by said replacement/omission("non-essential") (Morrison et al., Chemical Biology 5:302-307,

2001). Another option for testing the importance of various peptides is by the use of site-directed mutagenesis. Other Structure-Activity-Relationship (SAR) techniques may also be used.

An alanine scan replaces WT residues with alanine and generally measures loss of function. The technology referred to herein as amino acid-scanning has been used to precisely identify those amino acids directly involved in the active sites of some enzymes and receptors (see, e.g., Becl-Sickinger et al. (1994) Eur. J. Biochem. 223:947-958; Gibbs et al. (1991) J. Biol. Chem. 266:8923-8931; Matsushita et al. (2000) J. Biol. Chem. 275:11044-11049). The resulting polypeptides are shown below.

	Peptide Sequence
SEQ ID NO: 16	c(ACNTATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)
SEQ ID NO: 17	c(KCATATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)
SEQ ID NO: 18	c(KCNAATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)
SEQ ID NO: 19	c(KCNTAACVLGRLSQELHRLQTYPRTNTGSNTY-NH2)

Synthetic analogs based on SEQ ID NO: 4 incorporating the unnatural amino acids in AC 2389 (SEQ ID NO: 5) can also been synthesized. One example is SEQ ID NO: 21: Isocap-STAVLGRLSQELHRLQTYPRTNTGSNTY-NH_2.

Example 4: Food Intake Analysis of Additional Polypeptides

Additional polypeptides are identified for analysis. These additional polypeptides are used in food intake studies as described above. The results of the assays are shown in the Table below.

	Peptide Sequence	FOOD INTAKE(min)			
		30	60	120	180
SEQ ID NO: 10	c(KCNTATCVLGKLSQELHRLQTYPRTNTGSNTY-NH2)	-42	-31	-34	-30
SEQ ID NO: 11	c(KCNTATCVLGRLSQELHRLQTLPRTNTGSNTY-NH2)	-52	-47	-38	-35
SEQ ID NO: 12	c(KCNTATCVLGRLSQELHRLQTYPPTNTGSNTY-NH2)	-43	-39	-37	-32
SEQ ID NO: 13	c(KCNTATCVLGRLSQELHRLQTYPRTNVGSNTY-NH2)	-66	-53	-42	-32
SEQ ID NO: 14	c(KCNTATCVLGRLSQELHRLQTLPPTNVGSNTY-NH2)	-40	-33	-31	-28
SEQ ID NO: 15	c(KCNTATCVLGRLANFLHRLQTYPRTNTGSNTY-NH2)	-52	-36	-28	-33
SEQ ID NO: 16	c(ACNTATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	-67	-59	-37	-43
SEQ ID NO: 17	c(KCATATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	-26	-29	-30	-28
SEQ ID NO: 18	c(KCNAATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	-42	-30	-30	-25

SEQ ID NO: 19	c(KCNTAACVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	-2	-7	-16	-14
SEQ ID NO: 20	c(CANLSTCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	-25	-25	-28	-30
SEQ ID NO: 21	Isocap-STAVLGRLSQELHRLQTYPRTNTGSNTY-NH2	-9	-21	-30	-31
SEQ ID NO: 22	c(CSNASTCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	9	-5	-18	-18
SEQ ID NO: 23	c(CSNLATCVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	-11	-20	-31	-30
SEQ ID NO: 24	c(CSNLSACVLGRLSQELHRLQTYPRTNTGSNTY-NH2)	8	0	-19	-12
SEQ ID NO: 25	c(KCNTATCVLGRLSQELHKLQTYPRTNTGSNTY-NH2)	-40	-34	-35	-34
SEQ ID NO: 26	c(KCNTATCVLGRLSQELHRLQTYPRTNTGSGTP-NH2)	-29	-34	-38	
SEQ ID NO: 27	c(KCNTATCATQRLSQELHRLQTYPRTNTGSGTP-NH2)	7	-3	-6	

Example 5: Activity of ORPs on Weight Reduction and Caloric Intake

Male C57B1/6 mice (4 weeks-old at start of study) are fed high fat (HF; 58% of dietary kcal as fat) or low fat (LF; 11% of dietary kcal as fat) chow. After 7 weeks on chow, each mouse is implanted with an osmotic pump (Alzet# 2004) that deliveres 2.9 nmol/kg/day of either the vehicle, calcitonin, AC2221 (SEQ ID NO: 2), AC163231 (SEQ ID NO: 10), or AC163234 (SEQ ID NO: 13) continuously for 4 weeks. Body weight and food intake are measured weekly (Surwit, Feinglos, et al Metabolism--Clinical and Experimental 44: 645-651, 1995).

Figure 1 shows that the polypeptides administered produced a decrease in caloric efficiency (measured as body weight gained/kcal consumed) in diet-induced obese (DIO) mice.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

CLAIMS

What is claimed is:

1. A method of reducing weight in a subject in need of weight reduction or desirous thereof, comprising, administering a pharmaceutically effective amount of an obesity related polypeptide of SEQ ID NO: 1.

ABSTRACT

The present invention relates to obesity related polypeptides (ORPs), related nucleic acids, expression constructs, host cells, and processes production of the obesity related polypeptides. The ORPs of the invention include one or more amino acid sequence modifications. In addition, methods and compositions are disclosed to treat and prevent metabolic disorders such as obesity, diabetes, and increased cardiovascular risk.

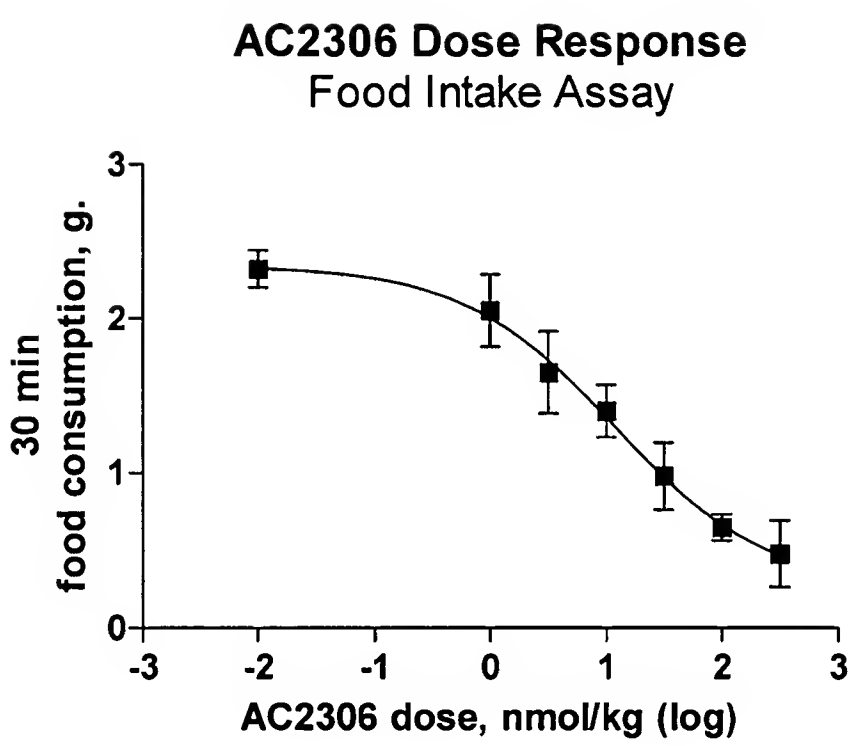
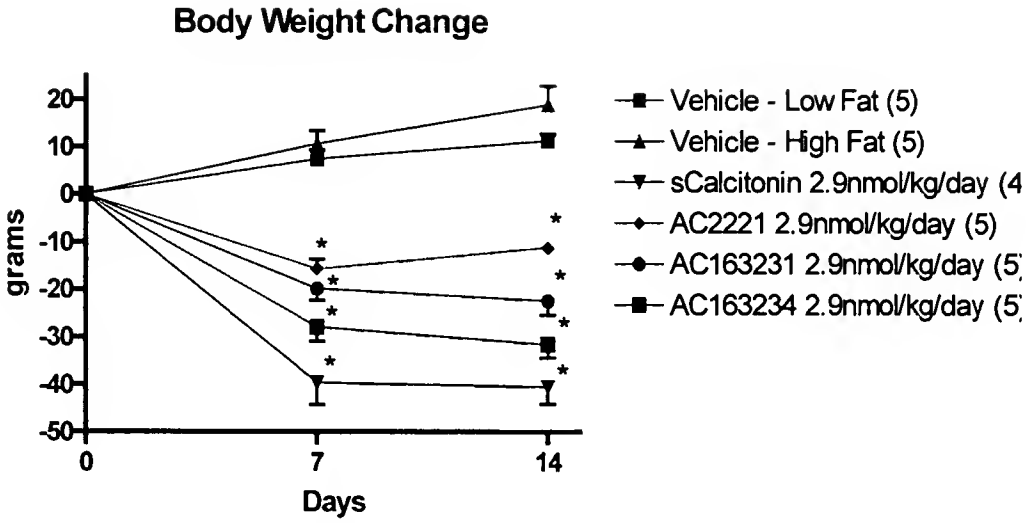
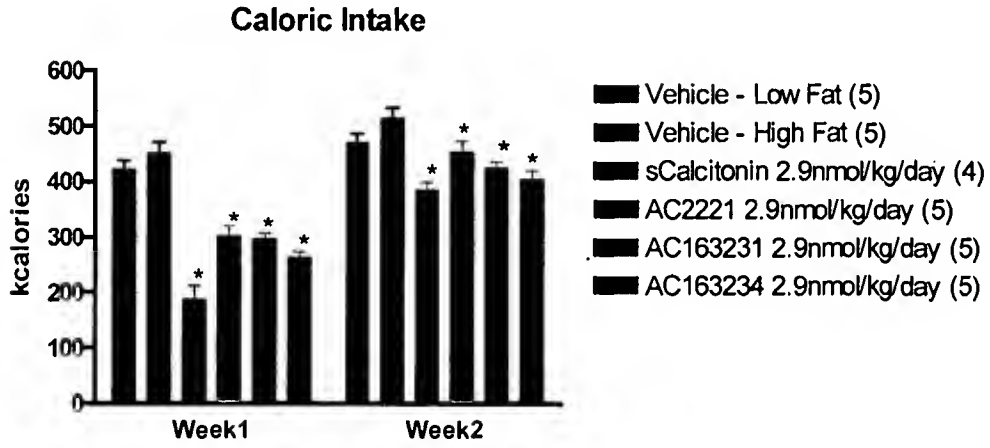

AC2306 Dose Response
Food Intake Assay
30 min food consumption, g.
3
2
1
0
-3
-2
-1
0
1
2
3
AC2306 dose, nmol/kg (log)

Figure 1

Figure 2





*P < 0.05 compared to respective vehicle-high fat group

SEQUENCE LISTINGS

SEQ ID NO: 1: AC0062:

KCNTATCATQ RLANFLVRSS NNLTNVGSNT Y-NH2

SEQ ID NO: 2: AC2221:

KCNTATCATQ RLANELVRLQ TYPRTNVGSN TY-NH2

SEQ ID NO: 3: AC2306:

CSNLSTCVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 4: AC2307:

KCNTATCVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 5: AC2389:

Isocap-STAVL-(Aib)-K(formyl)-LSQEL-(Aib)-K(formyl)-
LQTYPRTNTGSGTP-NH2

SEQ ID NO: 6: AC2607:

KCNTATCATQ RLANALVHSS NNFGAILPST NVGSNTY-NH2

SEQ ID NO: 7: AC3052:

KCNTATCATA RLAAFLARSS GY-NH2

SEQ ID NO: 8: AC3071:

KCNTATCATQ RLANFLVHSG NNFGAILSST NVGSNTY-NH2

SEQ ID NO: 9: AC3098:

CNTATCATAR LAAFLARS-NH2

SEQ ID NO: 10: AC163231:

KCNTATCVLG KLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 11: AC163232:

KCNTATCVLG RLSQELHRLQ TLPRTNTGSN TY-NH2

SEQ ID NO: 12: AC163233:

KCNTATCVLG RLSQELHRLQ TYPPTNTGSN TY-NH2

SEQ ID NO: 13: AC163234:

KCNTATCVLG RLSQELHRLQ TYPRTNVGSN TY-NH2

SEQ ID NO: 14: AC163235:

KCNTATCVLG RLSQELHRLQ TLPPTNVGSN TY-NH2

SEQ ID NO: 15: AC163237:

KCNTATCVLG RLANFLHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 16: AC163245:

ACNTATCVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 17: AC163246:

KCATATCVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 18: AC163247:

KCNAATCVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 19: AC163248:

KCNTAACVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 20: AC163249:

CANLSTCVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 21: AC163250:

Isocap-STAVLGRLS QELHRLQTYP RTNTGSNTY-NH2

SEQ ID NO: 22: AC163259:

CSNASTCVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 23: AC163260:

CSNLATCVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 24: AC163261:

CSNLSACVLG RLSQELHRLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 25: AC163262:

KCNTATCVLG RLSQELHKLQ TYPRTNTGSN TY-NH2

SEQ ID NO: 26: AC163276:

KCNTATCVLG RLSQELHRLQ TYPRTNTGSG TP-NH2

SEQ ID NO: 27: AC163278:

KCNTATCATQ RLSQELHRLQ TYPRTNTGSG TP-NH2